

# 20.7.2015

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MONDAY, 7/20

Petra, Tamannae

Made new 1:10 dilutions of P001 and P015.

- 2 ul primer to 18 ul water

Made new 1 ng/μl template dilution of CAR part 1.

- 2 μl DNA stock and 18 μl H<sub>2</sub>O

Gradient PCR for CAR part 1

- 12 tubes per gradient, 25 ul reaction mix per tube

Reaction mixes (13x25 ul = 325 ul)

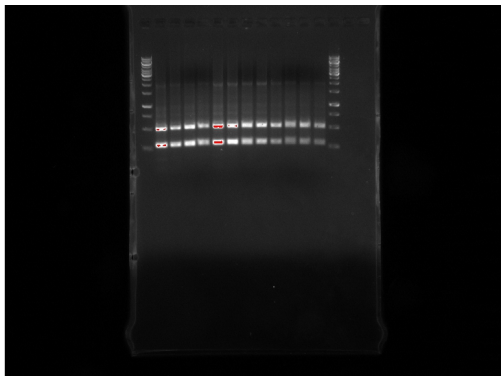
CAR part 1

162,5 ul 2x Mastermix  
16,25 ul 10μM P001  
16,25 ul 10μM P015  
13 ul 1:10 CAR part 1 template dilution  
117 ul water

Ran gradient PCR according to the protocol with 63-72 C gradient.

Made 1,2 % agarose gel with ETBR and ran 5 ul samples of each CAR1 gradient PCR reaction with 1 ul LD. Used O'GeneRuler 1 kb DNA ladder. The result: No correct amplicon was amplified.

Geldoc\_2015-07-20\_17hr\_43min\_GPCR\_CARpart1.jpg



Made 1:10 dilutions of P016, P017 and Car part 2: 1 ul DNA to 9 ul water.

PCR for CAR part 2

25 ul reaction mix for Ycia-sfp-CAR part 2

As in protocol (p016 + p017)

+ 1 ul 1:10 Ycia-sfp-CAR part 2 dilution

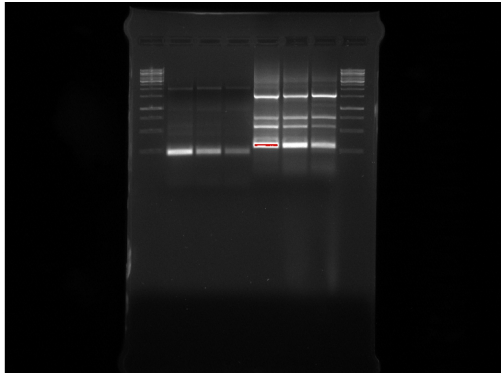
+ 9 ul water

Made 1,3 % agarose gel with EtBr and ran PCR products of AtoB part 2 and CAR part 3 in the gel for 30 min with 120 V.

Pipetting order was 1. ladder 2 μl 2. A2 with 1 % DMSO 18 μl 3. A2 with 3 % DMSO 18 μl

4. A2 with 5 % DMSO 18 μl 5. C3 with 1 % DMSO 18 μl 6. C3 with 3 % DMSO 18 μl 7. C3 with 5 % DMSO 18 μl 8. ladder 2 μl

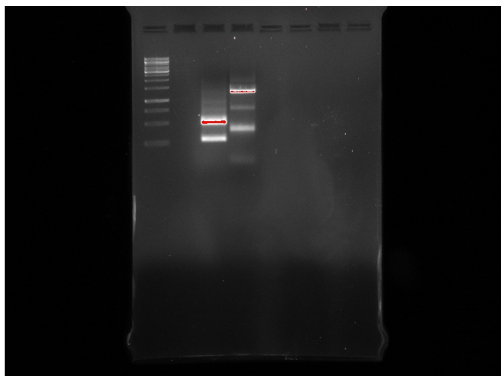
Geldoc\_2015-07-20\_12hr\_14min\_AtoB2\_CAR3.jpg



Gel purified AtoB2 and CAR3 following the kit protocol. Added 255,6  $\mu$ l binding buffer to the AtoB 2 tube and 220  $\mu$ l to the CAR3 tube.

Made 1,3 % agarose gel with EtBr and ran CAR1 and AtoB1. Pipeting order was 1. ladder 2  $\mu$ l 2. empty 3. C1 18  $\mu$ l 3. A1 18  $\mu$ l

Geldoc\_2015-07-20\_17hr\_08min\_CAR1AtoB1\_18ul.jpg



Then gel purified AtoB1 following the kit protocol. Added 23,2  $\mu$ l binding buffer.

NanoDrop results of todays gel purifications are below:

Table1

Sample	DNA (ng/ $\mu$ l)	A260/A280
AtoB2	5,6	3,71
CAR3	21,7	1,47
AtoB1	1,7	1,65

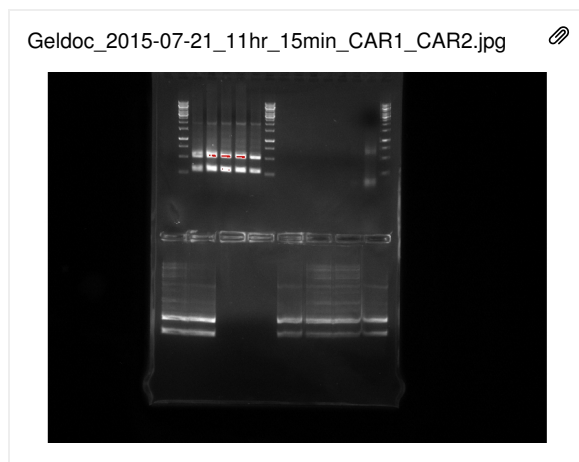
# 21.7.2015

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TUESDAY, 7/21

Petra, Tamannae

Made 1,3 % agarose gel with ETBR. Ran samples 5-8 of CAR part 1 gradient PCR made yesterday to purify DNA. Ran on gel 5 ul sample of CAR part 2 PCR reaction that was ran overnight to check the results. Used Thermo Fisher O'GeneRuler ladder. Ran the gel: 110 V, 25min



Results: No CAR part 2 in the PCR mixture. We didn't purify anything but we will continue by doing a gradient PCR for that part.

Purified CAR part 1 from the gel with GeneJET Gel Extraction Kit.

Nanodrop results:

- Concentration: 6,8 ng/ul
- A260/A280: 1,44

Made a gradien PCR for Ycia-sfp-CAR part 2:

Made CAR part 2 template dilution (1 ng/ul)

- 2 ul DNA to 18 ul sterilized water

Primer dilutions (10 ug/ul)

- 2 ul P016 to 18 ul sterilized water
- 2 ul P017 to 18 ul sterilized water

Reaction mix (13x25 ul =325 ul)

- 162,5 ul 2x Mastermix
- 16,25 ul 10 $\mu$ M P001
- 16,25 ul 10 $\mu$ M P015
- 13 ul 1:10 CAR part 2 template dilution
- 117 ul water

Ran gradient PCR accorcing to the protocol with 62-72 C gradient.

Diluted AH043 from the plate 4; 2L and transformed to TOP10 following mostly the protocol. However, 37 °C 1h incubation wasn't done (plasmid has the AMP resistant gene). Plated 20  $\mu$ l, 50  $\mu$ l and 150  $\mu$ l to AMP plates.

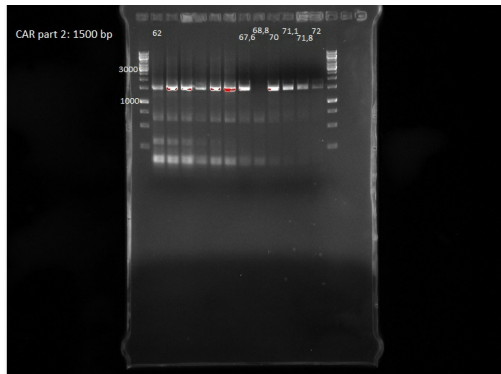
# 22.7.2015

WEDNESDAY, 7/22

Petra, Tamannae

Made a 1,3 % agarose gel with ETBR. Ran 5 ul samples (CAR 2) (with 1 ul LD) of CAR part 2 gradient PCR on the gel. Ran the gel 100V, 35 min. Used Thermo Fisher's Gene O'Ruler ladder.

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Gradient PCR results for CAR part 2

PCR purified CAR2 following the TS GeneJET PCR purification kit protocol. Purified samples 9, 10 and 11. Combined all three samples together to the same purification column.

NanoDrop result of CAR2:

Table1

Sample	DNA (ng/μl)	A260/A280
CAR 2	37,7	1,71

Transformation of AH043 was successful. Did o/n culture of AH043 in 2 ml LB with 2 μl AMP.

Did TOPO cloning for CAR part 1 and CAR part 3. Followed the protocol. Used 4 ul DNA to both reactions. Let the reaction proceed 10 minutes.

Transformed 2 ul of both TOPO cloning reactions to TOP10. Followed the protocol except that the incubation on ice lasted 40 minutes.

Plated 20 ul and 50 ul both transformants on kanamycin plates straight after heat shock. Incubated the rest of the reaction in 37 °C for 45 min. After incubation plated 20 ul and 50 ul of both transformants on kanamycin plates. Total amount of plates after transformation: 8

# 23.7.2015

THURSDAY, 7/23

Tamanna

Transformations of CAR1 & CAR3 with 45 min 37 °C incubation were successful. Transformations without incubation didn't create any colonies in the plates.

o/n culture of AH043 was red (so it's correct). So, did miniprep of AH043 following the NucleoSpin Plasmid EasyPure kit protocol. NanoDrop result of it:

Table1

Sample	DNA (ng/ul)	A260/A280
AH043	237,8	1,82

Did restriction of AH043 following the procol with enzymes XbaI & SpeI.

In istriction mix was:

2,5µl 10x NEB CutSmart Buffer

1,1 µl AH043

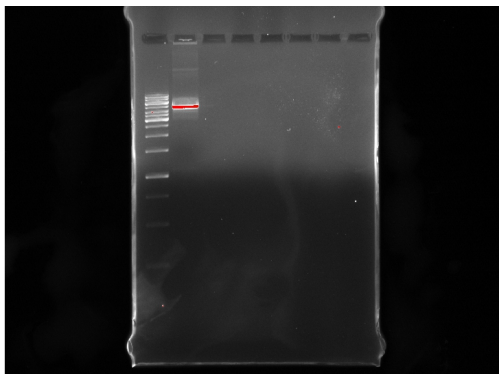
0,5µl XbaI

0,5µl SpeI

20,4 µl H<sub>2</sub>O

Made 1,3 % agarose gel with EtBr. Ran the gel for 40 min with 120 V.

Geldoc\_2015-07-23\_15hr\_07min.jpg



The restriction of AH043 wasn't successful, so did it again with the same mixture and restriction enzymes.

Picked 7 colonies from each plate which had the incubation for the Colony PCR for Ycia-sfp-CAR part 1 in TOPO and Ycia-sfp-CAR part 3 in TOPO. The picked colonies can be seen in Fig 2 (Ycia-sfp-CAR part 1, samples A1-A7) and Fig 3 (Ycia-sfp-CAR part 3, samples B1-B7). Followed the steps from the protocol of Colony PCR , and used 2 µl template DNA for the PCR. Followed the steps from the protocol Ycia-sfp-CAR part 1 and PCR for Ycia-sfp-CAR part 3. Made also line screening with kanamycin plate for the each colony, which samples were named A1-A7 and B1-B7. Incubated plate for 18 h.

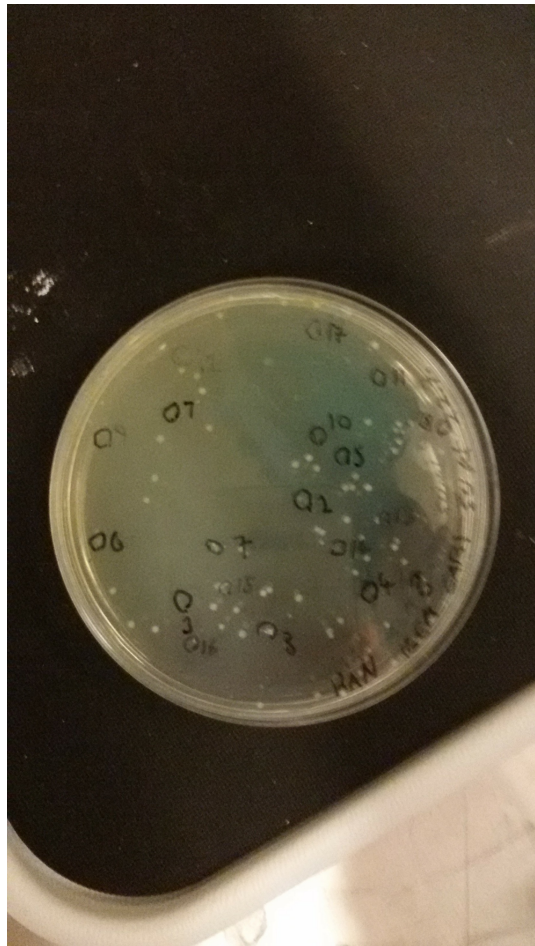


Fig 2. The screened colonies A1-A7 for the colony PCR.



Fig 3. The screened colonies B1-B7 for the colony PCR.

Made a colony PCR for Ycia-sfp-CAR part 1 and part 3:

Primer dilutions (10 ug/ul)

- 2 ul P001 to 18 ul sterilized water
- 2 ul P015 to 18 ul sterilized water
- 2 ul P011 to 18 ul sterilized water
- 2 ul P018 to 18 ul sterilized water

Reaction mix for each case

- 12,5 ul Q5 2x Mastermix
- 1,25 ul 10 $\mu$ M Primer 1
- 1,25 ul 10 $\mu$ M Primer 2
- 2 ul Template DNA from the colony
- 8 ul water

# 24.7.2015

FRIDAY, 7/24

Run a gel electrophoresis for yesterday's Colony PCR results (Ycia-sfp-CAR part 1 and PCR for Ycia-sfp-CAR part 3).

The PCR sample of 6B (Car part 3, 6th colony) seemed to contain half less volume than other samples so the pipetting error could have happened. Made 1,2 % agarose gel with ETBR and ran 5 ul samples of each colony

PCR with 1 ul LD and proceeded the gel electrophoresis (110V, 400A, 30 mins). Used O'GeneRuler 1 kb DNA ladder.

The gel results can be seen in Fig. 1 , where the samples 1-7 represents colonies A1-A7 (Ycia-sfp-CAR part 1) and 8-14 B1-B7 (Ycia-sfp-CAR part 3). Only the B6 (1542 bp) had the right sized DNA so it was purified with GeneJET PCR purification kit, following the protocol. The needed size for Ycia-sfp-CAR part 1 is 1945 bp and all the bands representing it are under 1kb. Also saved the gel sample for further gel purification. Fig. 2 shows the current location of B6 colony.

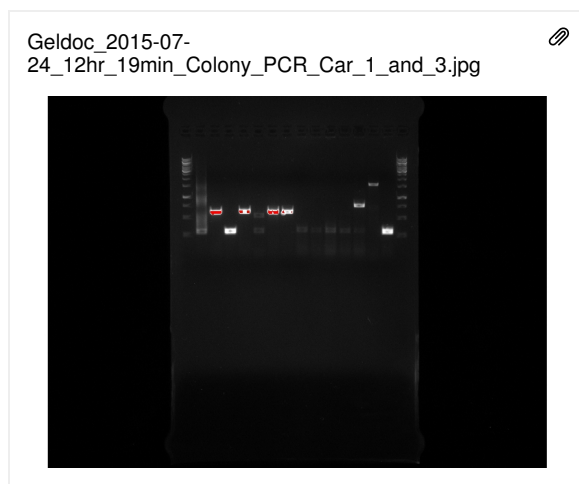


Fig 1. Gel electrophoresis results from the colonies of Ycia-sfp-CAR part 1 (samples 1-7) and Ycia-sfp-CAR part 3 (samples 8-14).

Did Colony PCR for colonies A8-A13 from the original plate to screen the right transformed plasmid. The original plate of TOPO transformants where the screened samples were taken can be seen in Fig 3. The samples are stored in a fridge at Heli's box. Made also line screening with kanamycin plate for the each colony, which samples were named A8-A13. Started incubation over the weekend at 15:00 at RT.

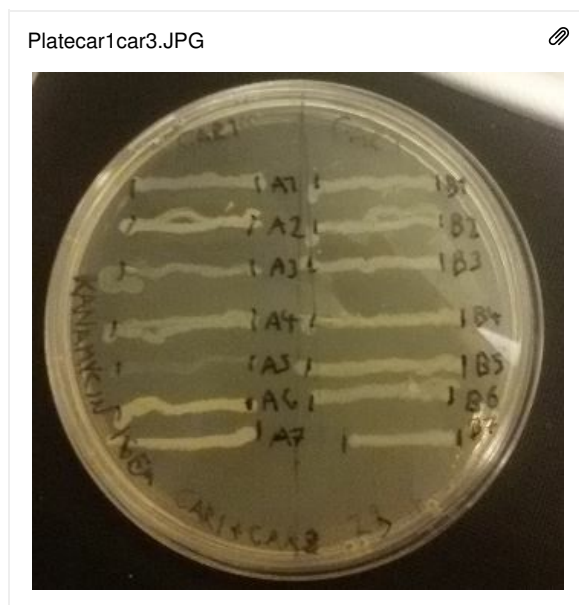


Fig 2. Colonies A1-A7 and B1-B7 to be screened



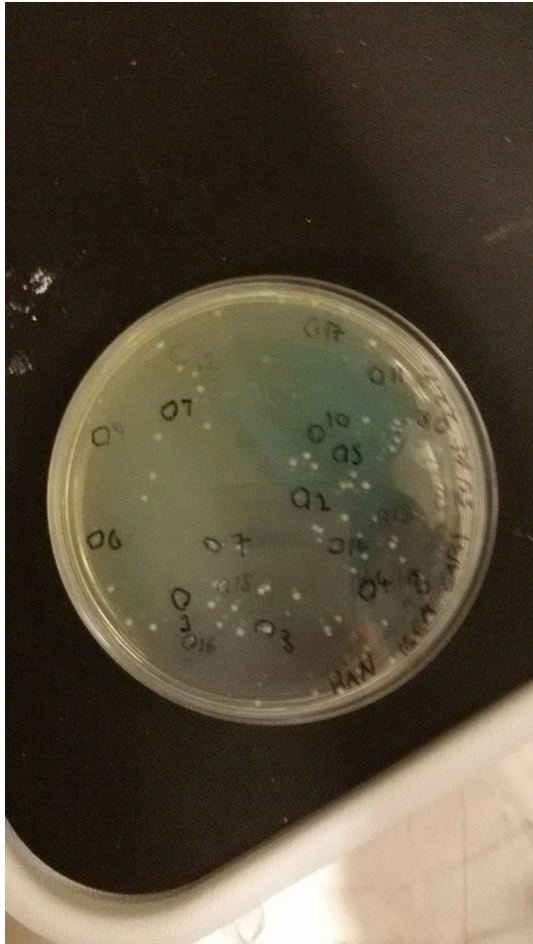


Fig 3. The screened colonies A8-A13 for the colony PCR.

The following NanoDrop results were obtained from GeneJET PCR purification:

Table1

Sample	DNA (ng/ $\mu$ l)	A260/A280
CAR3 from the PCR mix	1,1	1,45

Due to too low DNA concentration, the sample was discarded. Did the DNA purification from the gel with GeneJET Gel Purification kit and followed its protocols. Made a final stock volume of 20ul DNA elution and obtained the followed NanoDrop results for the gel purification:

Table2

Sample	DNA (ng/ $\mu$ l)	A260/A280
CAR3 from the Gel	22,7	3,07